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PATHOGENESIS AND PREVENTION OF ACUTE RENAL FAILURE(U)  
COLORADO UNIV HEALTH SCIENCES CENTER DENVER  
R W SCHRIER SEP 86 DAND17-85-C-5288

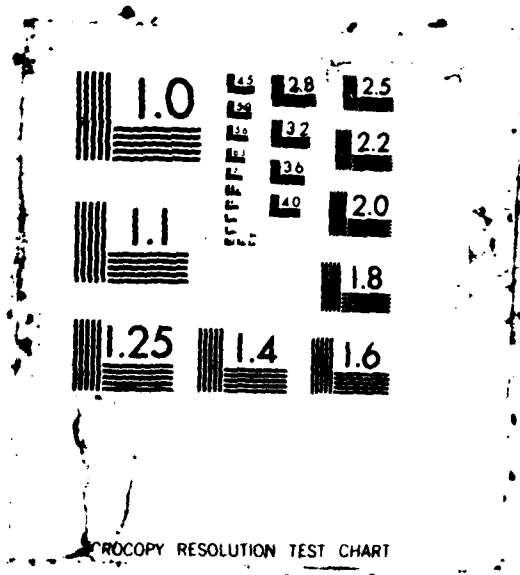
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PATHOGENESIS AND PREVENTION OF ACUTE RENAL FAILURE  
Annual Report

Robert W. Schrier, M.D.

September 1986

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-85-C-5288

University of Colorado School of Medicine  
Denver, Colorado 80262

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Freshly isolated rat proximal tubules and cultured rabbit tubules exposed to anoxia (100% N <sub>2</sub> ) die during reoxygenation unless pH is lowered or verapamil, nifedipine, trifluoropirazine or W-7 are administered. Thus, cell death and injury from increased calcium influx or calcium-calmodulin interactions are prevented. In addition, we have determined that various methods of lowering tissue ATP does not appear to compromise the baseline or subsequent recovery of cellular function after ischemia. Using NMR technology we have also followed renal tissue metabolism during and after hypotensive shock. The efficacy of various treatment protocols in shock will be evaluated in the coming year.		

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SUMMARY

During the first year of this contract (09/03/85-09/02/86), the protective effects of verapamil and nifedipine on preventing anoxia induced renal uptake of calcium were demonstrated and correlated with improved morphology and reduced LDH release. These calcium channel blockers (CCB) did not improve post-anoxic ATP levels which is similar to the observations of Shapiro and Chan who showed that improvement in renal function in the isolated perfused kidney, made anoxic, does not depend on cellular ATP levels being normal. The CCB and calcium-calmodulin antagonists also minimized cell death in cultured rabbit nephron segments. Maintaining extracellular pH at 6.9 rather than 7.4 could also prevent the anoxia-induced increase in calcium influx and would prevent morphologic injury. Finally, the time course of renal cellular metabolic changes during and after hemorrhage was determined with in vivo NMR spectroscopy. These baseline studies serve the purpose of providing a control level of values against which therapeutic maneuvers can be examined.

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FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

<u>Heading</u>	<u>Page</u>
Report Documentation Page . . . . .	
Title Page . . . . .	1
Summary . . . . .	2
Foreword . . . . .	3
Table of Contents . . . . .	4
Body of Report . . . . .	5
Literature Cited . . . . .	7
Distribution List . . . . .	8



## BODY OF REPORT

### Project 1

In the first year of contract DAMD 17-85-C-5288, the first objective has been accomplished. We questioned whether different calcium entry blockers, nifedipine and verapamil, which prevent anoxic cell injury do so by direct inhibition of calcium influx (or, in some manner, enhance the rate of calcium efflux). Both verapamil and nifedipine reduce the membrane calcium flux rate and the membrane calcium compartment size, which would otherwise be increased by anoxia, in proximal tubules of the rat. Furthermore, it was observed that anoxic tubules actually have increased rates of calcium efflux and that verapamil does not increase this rate any further. For reasons as yet unexplained, the morphology of nifedipine treated anoxic tubules did not measurably improve although verapamil treated anoxic tubules did demonstrate a reduced level of injury (compared to anoxia alone). Lactate dehydrogenase (LDH) activity in the extratubular fluid (media) was also lower in verapamil treated anoxic tubules (1).

We also determined that low extracellular pH (approx. 6.9) would prevent the increases in membrane and intracellular calcium flux and compartment size normally associated with anoxia. Preliminary results suggest further that such anoxic tubules already "protected" by anoxia are not benefited further by the additional administration of verapamil. These studies are continuing. In these pH protected tubules no measurable increase in cellular adenine nucleotides was observed even though morphology and LDH release were markedly improved (2).

Finally, we also tested whether calmodulin blocking drugs such as trifluoroperazine and W-7, added during reoxygenation, could influence survival of cultured rabbit tubules exposed to anoxic conditions. These drugs prolonged survival of nephron segments exposed to 45 min of  $N_2$  gas at  $25^\circ C$ . Doses of verapamil ( $5 \times 10^{-8}$  and  $5 \times 10^{-7}$  M) were also protective when added after anoxia (52 and 60%, respectively) and nifedipine at  $10^{-8}$  M (but not at  $10^{-7}$  M) was also protective (33% survival of tubules at 5 hr post-anoxia) (3).

### Project 2

Phosphate restriction has been shown to be protective against functional deterioration in several animal models of chronic renal failure. We therefore examined the effects of phosphate depletion accomplished through dietary phosphate restriction on the course of ischemic acute renal failure. In vivo, rats that were phosphate depleted had a more severe course of acute renal failure following a 40 min pedicle clamp. This finding has been shown to be independent of changes in serum calcium. However, when ischemia and reflow occurred in the isolated perfused kidney, there was no difference in functional recovery between phosphate depleted rats reperfused with no phosphate media and normal rats reperfused with normal phosphate media.

Based on these results, studies were performed studying intracellular phosphate depletion induced by glycerol or fructose added to the perfusion media in the isolated kidney. These maneuvers resulted in marked lowering of tissue levels of ATP (which has been described by others) but were not associated with significant differences in basal renal functions or recovery or renal function after ischemia (4). Longer term studies in vivo with fructose employing  $^{31}P$  NMR to measure intracellular free inorganic phosphate and ATP has confirmed these findings. (This work is being prepared for submission for publication.)

These studies suggest that intracellular phosphate depletion is not in and of itself deleterious to renal recovery from ischemia but that systemic

phosphate depletion through other mechanisms does worsen rather than ameliorate the course of in vivo ischemic acute renal failure in the rat. These mechanisms require further clarification but may be related to hemodynamic changes or changes in oxygen delivery that occur as consequences of systemic phosphate depletion.

Studies with  $^{31}\text{P}$  NMR were initiated this year. Renal tissue concentrations of ATP, inorganic phosphate and sugar phosphates and intracellular pH were established for normal rats as well as rats suffering from various degrees of hypotension and various periods of clamp ischemia. Hemorrhagic hypotension resulted in progressive decreases in intracellular ATP concentration, increases in sugar phosphate and inorganic phosphate concentrations and decreases in intracellular pH that correlated nicely with the degree and duration of hypotension. Ischemia resulted in similar but far more rapid changes. Recovery from ischemia resulted in a normalization of these values that was complete and rapid for short periods of ischemia but much slower for longer periods of ischemia. Tissue ATP was only 25% of control values 120 min after release of renal artery clamps applied for 60 min. Infusion of atriopeptin III intravenously at  $0.2 \mu\text{g/kg/min}$  resulted in a more rapid regeneration of ATP to 60% of control values by 120 min. This was accompanied by more rapid functional recovery both over the first several hours as well as the first 48 hr following ischemia (5). Further work with other protective maneuvers employing  $^{31}\text{P}$  NMR is planned for the coming year.

Calcium channel blockers were studied in several models of renal preservation. We had previously shown that verapamil was protective against both warm and cold ischemic injury in the isolated perfused kidney (Shapiro et al, Transplantation 1985). We studied emopamil, a new, potent calcium channel blocker (Knoll Pharmaceuticals) for protective effects against a combination of warm and cold ischemia mimicking a transplant setting. Indeed this agent tested quite favorably (6). Verapamil was tested for protective effects using continuous cold perfusion as the method of organ preservation. Renal functional performance following 24 hr of cold perfusion was improved by the addition of verapamil to the cold perfusate. (This work will be submitted to Transplantation for publication.)

#### Conclusion and Recommendation

These varied experimental maneuvers suggest that increased calcium influx is involved in the renal injury seen after ischemia or anoxia or under severe hypoxic conditions as might occur in hemorrhage. Even under conditions in which ATP depletion is quite rapid (i.e. total ischemia) as opposed to the slower depletion (i.e. hemorrhage, cold perfusion), it is clear that calcium channel blockers and calmodulin antagonists at the correct concentration given shortly after injury will attenuate the development of cell injury and death and speed functional recovery.

Further in vivo studies of the route, timing and duration of administration should be performed to establish animal model guidelines that might be amenable to clinical studies evaluating the efficacy of such therapy in humans. The side effects of these therapies such as peripheral vasodilation, diuresis and volume depletion, among others, can easily be observed in such animal models and corrected, if necessary, prior to having to deal with them in the clinical setting.

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